Ligation

Materials and Equipment

- Microtube Rack
- Micropipette
- Micropipette tips
- Microtubes (0.6 ml)
- Thermocycler

Reagents

- Nuclease-free water
- DNA
- 10X T4 DNA Ligase Buffer
- T4 DNA Ligase

Methodology

1. For a 20 µL reaction. Add in the following order:
2. 11 µL nuclease-free water
3. 4 µL Insert
4. 2 µL Receiving plasmid
5. 2 µL 10X T4 DNA Ligase Buffer
6. 1 µL T4 DNA Ligase
7. Incubate at room temperature (20˚C- 25˚C) for 2 hours
8. Heat inactivate at 80˚C for 20 minutes
9. Transform 10 µL ligation into 50 µL of competent cells
10. Store the rest at -20˚C